

ORIGINAL CONTRIBUTION

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Comparison of total phenols, flavonoids and antioxidant activity of various crude extracts of *Hyoscyamus gallagheri* traditionally used for the treatment of epilepsy

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Abstract

Background: Most of the locally available medicinal plant is used as medicine through different ways since the ancient time. *Hyoscyamus gallagheri* (*H. gallagheri*) is one of the rare medicinal plant which is indigenous to Oman as well as Gulf countries. The main target of this study is to estimate the total phenols and flavonoids content and evaluate the antioxidant activities of the aerial parts extracts which were collected from the southern part of Oman.

Methods: Initially, the powder of aerial parts of the selected plant was extracted with a polar solvent. The prepared extract was the successive fractionation by different polarities of solvents. All the extracts were used for the estimation of total phenols and flavonoids content by common Folin–Ciocalteu reagent (FCR) and calorimetric methods. In addition, the extracts were evaluated for their antioxidant activity by spectroscopic technique through α,α -diphenyl-picrylhydrazyl (DPPH) method.

Results: In the present experiment, the ethyl acetate extract contains the highest amount of phenols and the lowest in hexane extract. However, the highest amount of flavonoids was obtained from butanol extracts and the lowest in water aerial extract. In addition, the highest antioxidant activity showed in the ethyl acetate aerial extract and the lowest was in the hexane aerial extract. The other aerial extracts also showed the similar antioxidant activity comparable to Gallic acid standard.

Conclusion: It was also observed that there is a significant correlation between the antioxidant and total phenols. Therefore, the best activity aerial extract might be used for the treatment of human diseases in near future.

Keywords: *Hyoscyamus gallagheri*, Rare medicinal plant, Phenol, flavonoids, Antioxidant activity

Introduction

The search of relief from illness, through application of medicinally active plant crude drugs, has its origins in the distant pre-historic past. Egyptian medicine was flourishing several thousand years ago. The oldest records showed that the medicinal plant used as tablets was discovered in Mesopotamia and documented the ways of using hundreds of plant species. Still, now most of them are used to treat the human diseases [1].

Plant medicines are significant source of pharmaceuticals through several ways, which includes i) pure active compounds in their native form that are difficult or expensive to synthesize, ii) active compounds which have been modified to reduce toxicity and improve efficacy, iii) models for development of synthetic analogs with similar biological activities, and iv) inactive or poorly-active, but novel, chemical skeletons which promise interesting discoveries through modification [2, 3]. In addition, it is to contribute to pharmaceutical development, however, studies of the biological activity and chemistry of traditionally-used medicinal crude drugs should contribute to the rationalized use

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of these plants by the human population that depends on them for daily, front-line health care [4, 5].

Another vital role played by natural products as therapeutic materials is in the herbal industry in the U.S., as well as in European therapeutics markets where herbal drugs are sold and prescribed as standardized medicines. Pharmacognosy is one of the disciplines that have most actively embraced the development and promotion of naturally derived therapeutic materials, and has been successfully applying modern techniques to the discovery, re-discovery, development and standardization of marketable therapeutics [4–6]. It has been noted that nearly 50% of United States prescriptions filled in 1967 include in the formulation a natural drug product component derived from either higher plant, microbial, animal or mineral sources; a prescription audit in 1991 reported similar results [7].

Hyoscyamus gallagheri (*H. gallagheri*) is a rare medicinal plant, indigenous to Oman as well as some Gulf countries, which belongs to the family of Solanaceae [8, 9]. It is a flowering perennial herb with woody stems (Fig. 1). The stem length is about 1 m with gray-green. The leaves are crowded start from the branches. The size of the leaves is 50–70 × 10–15 mm with fleshy and oval size and the flowers are yellow with purple marking. The flowering starts during the September. The fruits are brown and papery with capsule size. The chemistry of the studied plants (*H. gallagheri*) has not been studied yet. However, the other species and genus of this family were well studied and found to exhibit broad spectrum biological activities and also several antimicrobial and cytotoxic principles were isolated and characterized from the other species which belong to this family [10, 11]. Traditionally, the selected plant species are used in Oman for treatment of insanity, epilepsy, toothache, hypertension, snake and scorpion bites [9, 12]. The extract or juice of leaves is used as a hair tonic for promoting hair growth [11, 12]. In this context, the present study is undertaken to estimate the amount of phenols and flavonoids contents and antioxidant activity of the aerial parts of different polarities extracts of *H. gallagheri*.



Fig. 1 Plant picture

Materials and methods

Chemicals, α,α -diphenyl-picrylhydrazyl (DPPH), gallic acid, Folin-Ciocalteu reagent (FCR) were bought from E. Merck (Germany). The solvents used for extraction were methanol, hexane, butanol, chloroform, dimethyl sulphoxide (DMSO), NaNO_3 , NaOH , and other general chemical and solvents were bought from Sigma-Aldrich Company Limited. The remaining other chemicals used in this experiment were of analytical grade.

Plant sample

The aerial parts of *H. gallagheri* were collected in the month of September, 2018 from Dhofar and the collected plant was authenticated by local area people and Associate Professor Dr. Syed Abdullah Gilani at the Department of Biological Sciences, University of Nizwa, Nizwa, Sultanate of Oman. The samples were transported to the Laboratory, Nizwa University for further process.

Preparation of samples

The *H. gallagheri* aerial parts samples were washed to remove dust and foreign materials and then air dried at room temperature (25°C) for 7 days. The dried aerial parts of the plant (100 g) were pulverized into coarse powder (approximately 2 mm mesh) by using a blender. The coarse powder samples were kept in a safe place to avoid contamination.

Extraction procedure

To prepare extract, Soxhlet extraction method was used for the powder samples of *H. gallagheri* (100 g) with methanol (1 L) at 40°C over a period of time several days. The solvent was concentrated in vacuo at reduced pressure and temperature (40°C) to obtain the corresponding extracts (9.7 g). The dried extract (7 g) was dissolved in water (200 ml) by using sonication and prepared different fraction by different polarities of solvent starting from nonpolar solvent through successive fractionation [13]. All separated fractions were evaporated at reduced pressure and temperature (40°C) to obtain the corresponding extracts (hexane (1.09 g), chloroform (1.99 g), ethyl acetate (2.17 g) and butanol (0.839 g)). The rest water part also evaporated by the same way and it is considered as a water extract (0.43 g). All polarities dry extracts including methanol extract (total six) were used for the determination of phenols, flavonoids and antioxidant activity using the selected standard protocols.

Total phenolic content (TPC) determination

Established FCR method with minor modification was used for the estimation of the phenol content of the aerial parts six extracts of *H. gallagheri* using gallic acid as an internal standard reported by Al-Saeedi et al. [14]. Five milligrams (5 mg) each fraction extract was dissolved in methanol in a

5 ml of volumetric flask. 10% FCR and 6% Na₂CO₃ were prepared by the addition of water in a 50 ml of volumetric flask. At first, three hundred microliters of each extract was taken in a 10 ml of glass test tube and instantly added 1.5 ml of 10% FCR solution. Then all the glass test tubes containing mixture were shaken and left for 5 min and added 1.5 ml of 6% Na₂CO₃ solution. All the experimental glass tubes were incubated in a dark place for 2 h at room temperature. Simultaneously, the gallic acid standard at various concentrations (200, 100, 50, and 25 µg/ml) were prepared in the established protocol as described in extracts. Later on, all plant aerial extracts and gallic acid standard solutions were measured absorbance against the blank as methanol at 760 nm with a UV/Visible spectrophotometer (UV-1800, Shimadzu, Japan). The phenol content of all different extracts was estimated by using the gallic acid standard calibration curve and the obtained results of total phenols were expressed as microgram of gallic acid equivalent (GAE) per 1 g of dry extract. All the measurement for the determination of phenol content of each extract was carried out in triplicate.

Determination of total flavonoid content (TFC)

Established modified AlCl₃ calorimetric method was used in this present experiment to estimate the flavonoid content in the aerial parts of six different extracts of *H. gallagheri* as previously reported by our group [14]. Four milligrams of each extract were dissolved in 4 ml of methanol in a 5 ml of volumetric flask. 5% NaNO₃, 5% NaOH and 7% AlCl₃ solution were prepared by using water in a 25 ml of volumetric flask. First of all, three hundred microliters of each extract were taken in a sealed glass vial and added 75 µl of 5% NaNO₃ and left for 5 min at room temperature. Later on 1.25 ml of AlCl₃ and 0.5 ml NaOH were added to each vial. Then it was sonicated and incubated for 5 min at room temperature. Simultaneously, the quercetin standard at different concentrations (200, 100, 50, and 25 µg/ml) were prepared in the same protocol as described in extracts. After incubation, the absorbance of all working solution and standard solution was measured against methanol blank at 510 nm. The flavonoids content of all different extracts was estimated by using the quercetin standard calibration curve and the obtained results of flavonoids were expressed as microgram of quercetin equivalent (Qu) per 1 g of dry extract. All the measurement for the determination of flavonoids content in the extract was carried out in triplicate.

Evaluation of antioxidant activity

The antioxidant activity of the aerial extract of *H. gallagheri* was assessed according to method describe by Alabri et al. [15], with further modifications. In this experiment, the DPPH reagent was used as the source of producing free radicals. The absorbance of free radicals of all aerial extracts

at various prepared concentrations was measured at 520 nm to decolorize upon their reduction ability. In the current study, as a standard, antioxidant compound gallic acid was used. Different concentrations of each extract (400, 200, 100, 50, and 25 µg/ml) were formed according to dilution method. At first, three hundred microliters (300 µl) of each concentration of each polarity extract were placed in a 5 ml of the glass tube and added 1.2 ml DMSO solution. Then 2.7 ml of DPPH (3.3 mg in 100 ml of DMSO) reagent was poured into the glass tube. All them were mixed together and held in the dark for 1.5 h. The decolorization of the DPPH was measured as absorbance at 516 nm using a UV-Vis spectrophotometer. In this experiment, DPPH solvent is being used as blank. The antioxidant activity was calculated of each concentration extract through the percentage of inhibition by using the formula

$$\% \text{Inhibition} = \frac{\text{Ao} - \text{As}}{\text{Ao}} * 100$$

Where Ao is the Absorbance of control and As is the absorbance of the test sample.

Data analysis

The collected data in this experiment are expressed as means ± standard deviation (SD). The experimental data were statistically analysed and the significance of differences between samples was determined by SPSS version 10.0 software. Values of *p* < 0.05 were considered statistically significant.

Results

Plant extracts

The collected plant aerial parts were processed and extracted with methanol solvent. The methanol solvent was removed from the extract and dissolved in water and successively fractioned with various solvents starting from nonpolar solvent. The mass of various polarities extracts from the aerial parts of *H. gallagheri* is presented in Table 1. In this experiment, the methanol extract through the Soxhlet extractor and its derived fraction are presented in Table 1. The yield of methanol extract obtained from the aerial parts was 9.7%. After

Table 1 Yield of various polarities extracts from the aerial parts of *H. gallagheri*

Extract	Yield (gm)
Methanol	9.7
Hexane	1.09
Chloroform	1.99
Ethyl acetate	2.17
Butanol	0.83
Water	0.43

fractioned, the highest yield was obtained in ethyl acetate and the lowest in the water.

Total phenol content

Total phenol contents of various polarities extracts obtained from the aerial parts of *H. gallagheri* were estimated modified FCR method describe by Al-Saeedi et al. [14]. In this assay, the FCR phenol detected reagent reacts with phenolic compounds under basic conditions to form chromogens that can be detected at 750 nm. The total phenol content of six plant extracts ranges from 45.58–1000 µg GAE/1 g dry extract (Fig. 2). Among the six aerial various polarities extracts, the maximum amount of total phenols found in ethyl acetate and minimum amount was in hexane extract. The experimental results obtained from the various extracts are presented in Table 2.

Total flavonoids content

Established modified calorimetric method was used for the estimation the flavonoid contents of the aerial of six different extracts of *H. gallagheri* as previously reported by our group [14]. In this method, the applied flavonoids reagent reacts with desire flavonoids compounds under the applied conditions to form flavonoids complex that can be detected at 510 nm. The range of flavonoids contents of six plant extracts from 1.86–185.17 µg QU/1 g dry extract (Fig. 3). Among the six aerial various polarities extracts, the significant amount of total flavonoids found in butanol and the minimum amount was in the ethyl acetate extract. The experimental results obtained from the various extracts are presented in Table 3.

Table 2 Total phenol contents of various extracts of *H. gallagheri*

Extract	Absorbance	Total phenol µg GAE/1 g dry extract
Hexane	0.251	45.58 ± 0.29
Ethyl acetate	0.381	1000 ± 0.10
Chloroform	0.316	523.50 ± 0.62
Methanol	0.348	758.5 ± 0.11
Butanol	0.327	604 ± 0.23
Water	0.352	785 ± 0.46

TriPLICATE experiment

Evaluation of antioxidant activity

The antioxidant activity was measured according to the method developed by Alabri et al. [15], with further slight modifications. Almost all the crude extract showed the significant percentage of inhibition comparable to gallic acid. Among them, the highest inhibition was obtained ethyl acetate and the lowest was in hexane extract. The antioxidant as a percentage of inhibition is presented in Table 4 and Fig. 4.

Discussion

Native communities in the Sultanate of Oman utilize a plenty of indigenous medicinal plants since the ancient times as a primary health care. However, there are no proper herbalist documentation or biological studies on the indigenous plants existed in current literature.

More than three-quarters of the active constituents which are derived from plants presently available in global use to treat human diseases that have been partially discovered through established research steps on folk and ethnomedicinal uses. It is authoritative that ethno

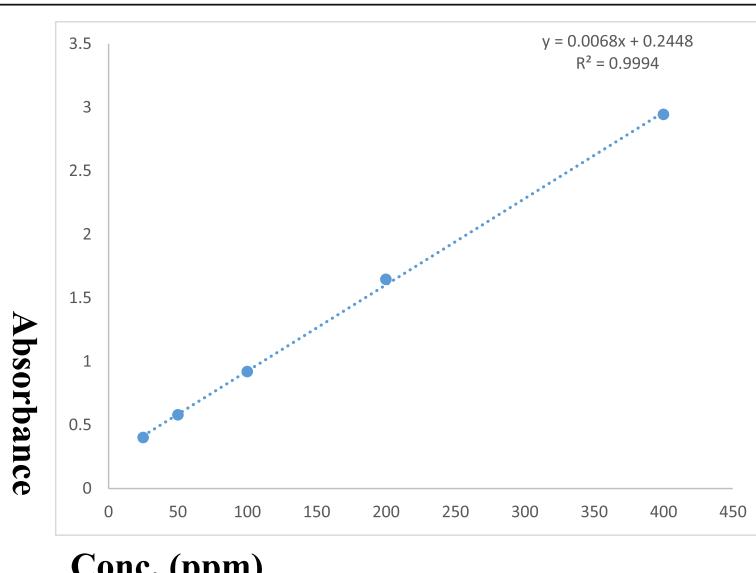
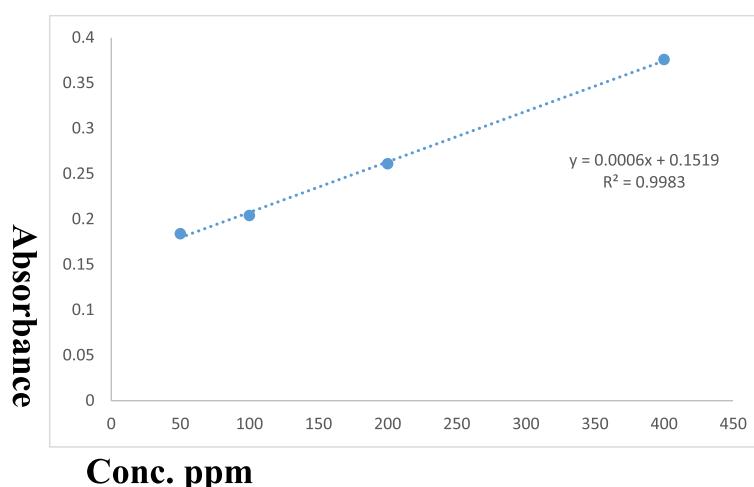


Fig. 2 Standard curve of gallic acid

**Fig. 3** Calibration curve for Quercetin standard

botanically based research to find active phytochemicals be inaugurated on a broad scale production [16].

This above data encouraged us to exploit the available natural resources in Oman towards the study on pharmacological and alleviation of different human oxidative stress. All kinds of oxidative stress plays a major part in the pathogenesis of numerous main human chronic diseases, such as cancer, heart diseases, diabetes and Alzheimer's condition. Also, transferable sicknesses are a vital concern in our present society due to the advent of manifold drug resistant strains of bacteria that cause millions of deaths worldwide. Therefore, the selected medicinal plant was undertaken to screen the bioactivities and phenols and flavonoids contents which were collected from the Southern part of Oman. The plant aerial parts were collected during the month of September, 2018. After collection, the samples necessary process was done in the preparation of the extract. Total of six extracts with increasing polarity were prepared according to the standard protocols mentioned above in the experimental sections.

Table 3 Total flavonoids content of various extracts of *H. gallagheri*

Extract	Absorbance	Total flavonoid µg QU/gm dry skin
Hexane	0.388	23.25 ± 0.11
Ethyl acetate	0.357	4.04 ± 0.23
Chloroform	0.456	29.30 ± 0.09
Methanol	0.356	20.44 ± 0.26
Butanol	0.49	185.17 ± 0.49
Water	0.336	1.86 ± 0.18

All data triplicate measurement

Medicinal plants are a gift of Allah and they contain a several groups of biologically active constituents, minerals, different types of vitamins and minor percentage of trace elements which is badly needed for human health. All the chemical constituents are a fascinating yet secretive group of several thousands of phytochemicals found in plant sources. Most of the phytochemicals are well known to be biologically active, which may exert a beneficial action on the human body. The mentioned phytochemicals have a wide range of positive action on the human body which include mainly the complex carbohydrates, flavonoids, terpenoids, proteins, several organic acids, alcohols and their corresponding complex, oils, tannins, saponins glycoside, glucosides and so on [17]. In addition, the primary and secondary compounds produced by plants to support their existence and biological effects. These above mentioned phytochemicals are being researched for nutrition and herbal medicine [18]. They have significant antioxidants, antimicrobial, antiviral and inspiration hormones, immune function, various inflammations, detoxification and other cell functions. Most of the natural sources, especially plant sources afford a source of biologically active compounds which are currently used as poisons, drugs or beneficial agents [19].

Total phenol content

The total phenols content assay of the aerial six extracts was performed according to a procedure established by Al-Saeedi et al. [14]. All extracts exhibit some amount of total phenol content. The maximum level of phenols was obtained in the ethyl acetate extract and minimum was in hexane extract and the following hierarchy: ethyl acetate>water>methanol>chloroform>butanol> hexane

Table 4 Antioxidant activity results of various polarities extracts of *H. gallagheri*

Extract	Concentration (ppm)	Absorbance (nm)	% Inhibition
Hexane	400	0.039	96.43 ± 0.09
	200	0.13	96.40 ± 0.21
	100	0.168	84.60 ± 0.42
	50	0.182	83.30 ± 1.08
	25	0.095	91.30 ± 0.36
Chloroform	400	0.029	97.30 ± 0.12
	200	0.074	93.20 ± 0.30
	100	0.132	55.50 ± 0.55
	50	0.153	86.00 ± 0.51
	25	0.121	88.90 ± 0.69
Ethyl acetate	400	0.021	98.07 ± 0.71
	200	0.024	97.80 ± 0.08
	100	0.094	91.30 ± 0.19
	50	0.129	88.10 ± 0.17
	25	0.096	91.20 ± 0.76
Butanol	400	0.018	51.30 ± 0.12
	200	0.532	98.60 ± 0.43
	100	0.015	97.00 ± 0.14
	50	0.022	97.40 ± 0.22
	25	0.032	97.07 ± 0.32
Water	400	0.028	97.07 ± 0.76
	200	0.135	87.60 ± 0.33
	100	0.470	56.90 ± 0.15
	50	0.286	73.80 ± 0.13
	25	0.212	89.02 ± 0.10
Gallic acid	400	0.037	98.13 ± 0.15
	200	0.038	98.09 ± 0.71
	100	0.038	97.80 ± 0.34
	50	0.039	97.50 ± 0.19
	25	0.040	97.01 ± 0.21

All experiment triplicates

extract (Table 2). The present phenols content results are unable to compare with other results due to the usability of the literature on the selected plant species. But, there are several reports available in the literature on the other species belong to this family. The total phenol content of other species also showed the average amount present in the plant extracts reported [20, 21].

Total flavonoids content

Similarly, total flavonoids content of the aerial six extracts of *H. gallagheri* was performed according to the procedure

established by Al-Saeedi et al. [14]. All extracts showed some amount of total flavonoids content. In our experiment, butanol extract has a significant amount of flavonoids compare to other prepared extract. But the minimum amount of flavonoids was obtained in water extract and the following hierarchy: butanol>chloroform>-hexane>methanol> ethyl acetate>water extract (Table 3). This result showed that most of the flavonoids were present in the butanol aerial extract. In addition, butanol is polar solvent and all of the flavonoids obtained in the butanol extract are polar in nature. The flavonoids content results are unable to compare with other results due to the usability of the literature on the selected plant species. But, several reports are available in literature on the other species belong to this family. Several authors reported on the other species belonging to the family and it was supported in our results [22, 23].

Evaluation of antioxidant activity

The determination of antioxidant activity of aerial various extracts of *H. gallagheri* was measured by the DPPH method developed by Alabri et al. [15], with further slight modifications. Almost all the crude extracts showed significant inhibition compared to gallic acid. Among them, the highest inhibition was obtained in ethyl acetate and the lowest was in hexane extract. The antioxidant as a percentage of inhibition is presented in Table 4 and Fig. 4. All crude extract basically reacts with DPPH and form a free radical and finally gradually decrease the colour of DPPH. The present experiment, all the aerial extracts from *H. gallagheri* were able to decolorize the DPPH colour. However, the ethyl acetate gave us the best results for decolorization that indicates the extract contains the maximum number of antioxidant compounds which can decolorize the colour rapidly of DPPH. We could not find any reports on antioxidant activity of the various polarities extracts of the selected plant species, however, there are several reports available on antioxidant activity screening belongs to this family's plant species [24–28]. Therefore, we are unable to compare our results to the other reports. However, our experimental data are consistent with the other species belonging to this family.

Conclusion

In our experimental, data showed that all the crude extracts from the aerial parts of *H. gallagheri* has a significant amount of total phenols and flavonoids contents through the methods of FCR and calorimetric methods. Among the prepared extracts, ethyl acetate contains the maximum total phenol content. Similarly, the butanol extract contains the maximum amount of flavonoids content. In addition, the antioxidant activity of the six prepared extracts showed also the good inhibition

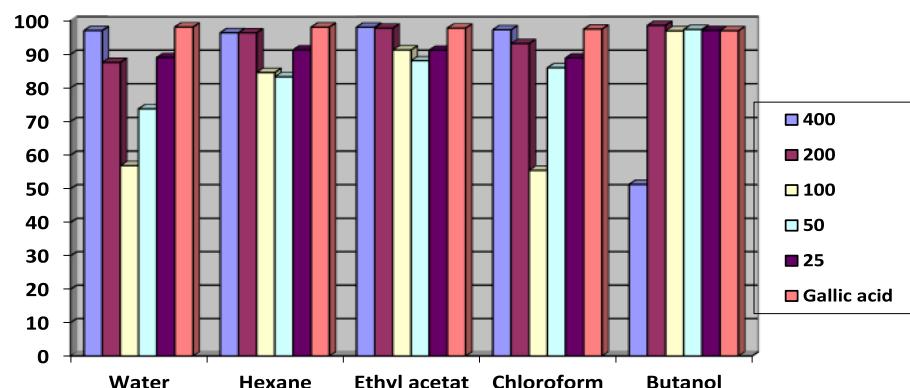


Fig. 4 Antioxidant capacity of different crude extracts from aerial samples of *H. gallagheri*

against the DPPH. The ethyl acetate extract showed the maximum activity among the various extracts. Our results also showed that there are very good correlation with the total phenol and antioxidant activity. Therefore, we might consider the highest activity extract as a natural antioxidant for the protection of different curable and incurable diseases.

Abbreviation

DMSO: Dimethyl sulphoxide; DPPH: α,α -diphenyl-picrylhydrazyl; FCR: Folin-Ciocalteu reagent; Na_2SO_4 : Sodium sulphate; NaNO_3 : Sodium nitrate; NaOH: Sodium hydroxide

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Authors' contributions

MAH prepared and submitted the manuscript. AMW reviewed and gave critical analysis of the manuscript. KS contributed to the work. Both authors read and approved the final manuscript.

Ethics approval

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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